PATENT
ATTORNEY DOCKET NO.: 056291-5137

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re PATENT APPLICATION of:

DAVIS et al.

U.S. Application No.: 09/869,925

Filed: July 9, 2001

FOR: COLCHINOL DERIVATIVES AS VASCULAR DAMAGING AGENTS

Group Art Unit: 1653

Examiner: Lukton, David

DECLARATION OF SUSAN ELIZABETH ASHTON

Susan Elizabeth Ashton, a citizen of Great Britain and having the post office address of Alderley Park, Macclesfield, Cheshire SK 10 4TG, Great Britain, hereby declares as follows:

1. I hold a MI Biol degree in Pharmacology from Stockport College of Technology.

2. I currently hold the position of Associate Team Leader for AstraZeneca (UK) Ltd, which position I have held since April 2000. I was the lead bioscientist on a research project with the aim of identifying selective vascular damaging agents for the treatment of cancer.

3. I am familiar with the specification of published PCT application WO 00/40529. I am informed that the text of the specification of subject U.S. Application No. 09/869,925 is the same as the text of this published PCT application. My page and line references herein will be to the published PCT application with the understanding that they will be the same in the subject U.S. application.

4. The specification of the PCT application sets forth at pages 50-53 various procedures for determining the potency and selectivity of the compounds disclosed in this

application to selectively damage newly formed tumour vasculature while leaving unaffected normal, mature vasculature. These procedures include the HUVEC detachment (assay (c) on page 52), which is an *in vitro* assay that measures the effects of compounds on the adherence of HUVECs to tissue culture plasticware, and the Hras5 necrosis model (assay (d) on page 53 of the specification), which is an *in vivo* assay that measures the relative effect of a compound in inhibiting tumour growth by assessing the relative area of necrosis of individual tumours taken from the test animals.

6. In my position as associate team leader I am familiar with the tests that were carried out on compounds exemplified in the present application using assay (c) and assay (d), and with the records of those test results that were maintained in the normal course of business of AstraZeneca. I have verified the *in vitro* and *in vivo* test data reported on the attached table of Appendix I against the test results documented in those records.

7. The table of Appendix I reports data for a representative sample of the compounds exemplified in the present application showing their activity as vascular damaging agents, including data from the human umbilical vein endothelial cell (HUVEC) detachment assay and the Hras5 necrosis model, which are the assays noted in paragraph 5 above and described at pages 52 and 53 of the specification. Certain of these compounds are identified as "prodrugs," that is, the exemplified compound is converted to the active moiety *in vivo*, after administration to a warm blooded animal. Thus, for these compounds, only data for the *in vivo* Hras5 model is included since it would be expected that these "prodrugs" would only have low activity in the *in vitro* HUVEC detachment assay. When establishing the Hras5 necrosis model no adverse events were observed either when dosing representative compounds to non-tumour bearing male nude mice or when dosing Appendix I examples to tumour bearing male nude mice during the 24 hour period of the assay.

8. These data demonstrate that the tested compounds inhibit tumour growth in an *in vivo* tumour model, and produce a vascular damaging effect as shown by an effect *in vitro* on

endothelial cells in the HUVEC detachment assay, which is translated to an anti-tumour effect *in vivo* in the Hras necrosis model.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.

S. Ashton

Susan Elizabeth Ashton

14/10/04

Date

Appendix I

Example No.	HUCEC Detachment Assay	Hras5 Necrosis Model
2	No data (prodrug)	9.3 ± 0.3 @ 100mg/kg IP
4	No data (prodrug)	3.8 ± 0.6 @ 100mg/kg IP
8	37%	No data
9	53%	0.3 ± 0.3 @ 100mg/kg IV
12	No data (prodrug)	1.0 ± 0.0 @ 100mg/kg IV
16	No data (prodrug)	7.3 ± 1.0 @ 100mg/kg IP
17	No data (prodrug)	4.0 ± 1.7 @ 100mg/kg IP
18	No data (prodrug)	6.8 ± 1.4 @ 100mg/kg IP
19	No data (prodrug)	0.0 ± 0.0 @ 50mg/kg IV
20	No data (prodrug)	0.3 ± 0.3 @ 100mg/kg IV
25	28%	No data
26	65%, 40%	No data
27	No data (prodrug)	0.5 ± 0.5 @ 100mg/kg IP
28	No data (prodrug)	6.0 ± 1.1 @ 50mg/kg IP
30	26%, 17%	No data
31	No data (prodrug)	0.3 ± 0.3 @ 100mg/kg IP
32	No data (prodrug)	0.8 ± 0.5 @ 100mg/kg IP
35	No data (prodrug)	0.3 ± 0.3 @ 100mg/kg IV
36	21%, 18%, 25%	0.3 ± 0.3 @ 50mg/kg IP
37	No data (prodrug)	0.5 ± 0.3 @ 100mg/kg IV
38	37%	0.3 ± 0.3 @ 100mg/kg IV
39	11%, 45%	1.8 ± 0.9 @ 100mg/kg IP
40	No data (prodrug)	6.0 ± 1.5 @ 100mg/kg IP
41	No data (prodrug)	6.3 ± 0.8 @ 100mg/kg IP
43	No data (prodrug)	1.0 ± 0.0 @ 100mg/kg IP
44	No data (prodrug)	0.8 ± 0.5 @ 100mg/kg IV
46	No data (prodrug)	2.3 ± 0.9 @ 100mg/kg IP
48	27%	No data
49	32%	No data
50	64%	No data
51	61%	No data
52	No data (prodrug)	3.3 ± 1.3 @ 50mg/kg IP
53	No data (prodrug)	3.0 ± 1.4 @ 100mg/kg IP

Huvec Detachment Assay – expressed as % cells detached compared to controls, compound concentration 100 μM. Where 2 values are quoted this relates to data from two different experiments

Hras Necrosis model – expressed as mean ± standard error of n=3-4 individual mice tumour necrosis scores.